

CLAIMS

1. Immunoglobulin comprising two heavy polypeptide chains sufficient for the formation of at least one complete antigen binding site, wherein the immunoglobulin is devoid of light polypeptide chains.

2. Immunoglobulin according to claim 1 obtainable by purification from serum of Camelids, wherein said immunoglobulin:
is not adsorbed by chromatography on Protein G Sepharose column;

is adsorbed by chromatography on Protein A Sepharose column;

has a molecular weight of around 100 Kd after elution with a pH 4.5 buffer (0.15 M NaCl, 0.58% acetic acid adjusted to pH 4.5 by NaOH); and

comprises heavy γ_2 polypeptide chains of a molecular weight of about 45 Kd, preferably 46 Kd, after reduction.

3. Immunoglobulin according to claim 1, obtainable by purification from serum of Camelids, wherein the immunoglobulin:

is adsorbed by chromatography on a Protein A Sepharose column;

has a molecular weight of about 100 Kd after elution of a pH 3.5 buffer (0.15 M NaCl, 0.58% acetic acid);

is adsorbed by chromatography on a Protein G Sepharose column and eluted with pH 3.5 buffer (0.15 M NaCl, 0.58% acetic acid); and

comprises heavy $\gamma 3$ polypeptide chains of a molecular weight of about 45 Kd, in particular between 43 and 47 Kd, after reduction.

4. Fragment of an immunoglobulin according to claim 1, wherein the fragment is selected from the following group:

- a fragment corresponding to one heavy polypeptide chain of an immunoglobulin devoid of light chains;

- fragments obtained by enzymatic digestion of the immunoglobulins of claim 1, especially those obtained by partial digestion with papain leading to the Fc fragment (constant fragment) and leading to FV_{HH}^h fragment (containing the antigen binding sites of the heavy chains) or its dimer $F(V_{HH}^h)_2$, or a fragment obtained by further digestion with papain of the Fc fragment, leading to the Fc' fragment corresponding to the C-terminal part of the Fc fragment;

- homologous fragments obtained with other proteolytic enzymes;

- a fragment of at least 10, preferably 20, amino acids of the variable region of the immunoglobulin, or the complete variable region, especially a fragment corresponding to the isolated V_{HH} domains or to the V_{HH} dimers linked to the hinge disulfide;

- a fragment corresponding to the hinge region of the immunoglobulin, or to at least 6 amino acids of this hinge region;

- a fragment of the hinge region comprising a repeated sequence of Pro-X; and

a fragment corresponding to at least 10, preferably 20, amino acids of the constant region or to the complete constant region of the immunoglobulin.

5. Immunoglobulin according to claim 1, wherein all or a part of the constant region of the immunoglobulin is replaced by all or part of the constant region of a human antibody.

6. Immunoglobulin according to claim 1, obtainable in prokaryotic cells, especially in *E. coli* cells, by a process comprising the steps of:

- (a) cloning in a Bluescript vector a DNA or cDNA sequence coding for the VH domain of an immunoglobulin devoid of light chain, obtainable for instance from lymphocytes of Camelids;
- (b) recovering the cloned fragment after amplification using a 5' primer containing an Xho site and a 3' primer containing the Spe site having the following sequence
TC TTA ACT AGT GAG GAG ACG GTG ACC TG;
- (c) cloning the recovered fragment in phase in an immuno PBS vector after digestion of the vector with Xho and Spe restriction enzymes;
- (d) transforming host cells, especially *E. coli*, by transfection with the recombinant immuno PBS vector of step (c); and
- (e) recovering the expression product of the V_{HH} coding sequence, for instance by using antibodies raised against the dromadary V_{HH} domain.

7. Hetero-specific immunoglobulins according to claim 1 obtainable by a process comprising the steps of:

obtaining a first DNA or cDNA sequence coding for a V_{HH} domain or part thereof having a determined specificity different from the specificity of the first DNA or cDNA sequence and comprised between the Spe and EcoRI sites;

digesting an immuno PBS vector with EcoRI and XhoI restriction enzymes;

ligating the obtained DNA or cDNA sequences coding for V_{HH} domains so that the DNA or cDNA sequences are serially cloned in the vector;

transforming a host cell, especially *E. coli* cell, by transfection; and

recovering the obtained immunoglobulins.

8. Immunoglobulin according to claim 1, obtainable by a process comprising the steps of:

obtaining a DNA or cDNA sequence coding for a V_{HH} domain or part thereof having a determined specific antigen binding site;

amplifying the obtained DNA or cDNA, using a 5' primer containing an initiation codon and a HindIII site, and a 3' primer containing a termination codon having a XhoI site;

recombining the amplified DNA or cDNA into the HindIII (position 2650) and XhoI (position 4067) sites of a plasmid pMM984;

transfecting permissive cells, especially NB-E cells, with the recombinant plasmid; and

controlling the expression, for instance by an ELISA assay with antibodies directed against a region of a V_{HH} domain; and

recovering the obtained products.

9. Nucleotide sequence encoding all or part of an immunoglobulin according to claim 1, which immunoglobulins comprise a peptide sequence selected from the group consisting of:

VTVSSGTNEVCKCPKCPAPELPGGPSVVFVFP,

VTVSSEPKIPQPPKPPQPPKPPQPEPECTCPKCPAPELLGGPSVFIFP

GTNEVCKCPKCP

APELPGGPSVVFVFP

EPKIPQPPKPPQPPKPPKPEPEECTCPKCP

APELLGGPSVFIFP

APELLGGPTVFIFPPKPKDVLSITLTP

APELPGGPSVVFVFPKPKDVLSISGRP

APELPGGPSVVFVFPKPKDVLSISGRP

APELLGGPSVFIFPPKPKDVLSISGRP

GQTREPQVYTTLA

GQTREPQVYTTLAPXRLEL

GQPREPQVYTTLPPSRDEL

GQPREPQVYTTLPPSREEM

GQPREPQVYTTLPPSQEEM

G G S V Q T G G S L R L S C E I S G L T F D

G G S V Q T G G S L R L S C A V S G F S F S

G G S - E Q G G G S L R L S C A I S G Y T Y G

G G S V Q P G G S L T L S C T V S G A T Y S

G G S V Q A G G S L R L S C T G S G F P Y S

GGSVQAGGSLRLSCVAGFGTS
GGSVQAGGSLRLSCVSFSFSS
WGQGTQVTVSS
WGQGTLVTVSS
WGQGAQVTVSS
WGQGTQVTASS
RGQGTQVTVSL

and/or,

ALQPGGYCGYGX - - - - - CL
VSLMDRISQH - - - - - GC
VPAHLGPGA ILDLKKY - - - - - KY
FCYSTAGDGGSGE - - - - - MY
ELSGGSCELPLLF - - - - - DY
DWKYWTCGAQTGGYF - - - - - GQ
RLTEMGACDARWATLATRTFAYNY
QKKDRTRWAEPREW - - - - - NN
GSRFSSPVGSTSRLES - SDY - - NY
ADPSIYYSLXIEY - - - - - KY
DSPCYMPTMPAPP IRDSFGW - - DD
TSSFYWYCTTAPY - - - - - NV
TEIEWYG CNLRTTF - - - - - TR
NQLAGGWYLDPNYWLSVGAY - - AI
RLTEMGACDARWATLATRTFAYNY
DGWTRKEGGIGLPWSVQCEDGYNY
DSYPCHLL - - - - - DV
VEYPIADMCS - - - - - RY

10. Process for the preparation of a monoclonal antibody according to claim 1, directed against a determined antigen, the antigen binding site of the antibody comprising heavy polypeptide chains, wherein the antibody is devoid of light polypeptide chains, said process comprising:

immortalizing lymphocytes, obtained for example from the peripheral blood of Camelids previously immunized with a determined antigen, with an immortal cell, and preferably with myeloma cells, in order to form a hybridoma;

culturing the immortalized cells formed; and

recovering the cells producing the antibodies having the desired specificity.

11. Process for the preparation of antibodies directed against determined antigens, comprising the steps of:

cloning into vectors, especially into phages and more particularly filamentous bacteriophages, a DNA or cDNA sequence obtained from lymphocytes of Camelids previously immunized with determined antigens, capable of producing an immunoglobulin according to claim 1;

transforming prokaryotic cells with said vectors in conditions allowing the production of the antibodies;

selecting the appropriate antibody by subjecting the transforming cells to antigen-affinity selection; and

recovering the antibodies having the desired specificity.

12. A peptide for coupling protein domains or a protein and a ligand, wherein the peptide comprises a repeated sequence Pro-X,

X being any amino acid and preferably Gln, Lys or Glu, the sequence containing at least 3 repeats of Pro-X.

13. Recombinant vector comprising a nucleotide sequence according to claim 9, wherein the vector is a plasmid, a phage especially a bacteriophage, a virus, a YAC, or a cosmid.

14. Recombinant cell or organism modified by a vector as claimed in claim 13.

15. A cDNA library comprised of nucleotide sequences coding for a heavy-chain immunoglobulin according to claim 1, obtained by performing the following steps:

- (a) treating a sample containing lymphoid cells, especially peripheral lymphocytes, spleen cells, lymph nodes or another lymphoid tissue from a healthy animal, especially selected among the Camelids, in order to separate B-lymphocytes;
- (b) separating polyadenylated RNA from other nucleic acids and components of the cells;
- (c) reacting the obtained RNA with a reverse transcriptase in order to obtain the corresponding cDNA;
- (d) contacting the obtained cDNA with 5' primers corresponding mouse V_H domain of four-chain immunoglobulins, which primer contains a determined restriction site, for example an XhoI site, and with 3' primers corresponding to the N-terminal part of a C_H2 domain;
- (e) amplifying the DNA;

- (f) cloning the amplified DNA in a vector, especially in a bluescript vector; and
- (g) recovering the clones hybridizing with a probe corresponding to the sequence coding for a constant domain of an isolated heavy-chain immunoglobulin.

16. A modified 4-chain immunoglobulin or a fragment thereof, the V_H regions of which have been partially replaced by specific sequences or amino acids of heavy chain immunoglobulins according to claim 1.

17. A modified 4-chain immunoglobulin or a fragment thereof, in which CDR loops of the region are linked to other parts of V region by introduction of paired cysteines, in particular in which the CDR_3 loop is linked to the FW_2 or CDR_1 , and more especially where the cysteine of the CDR_3 of the V_H is linked to a cysteine in position 31 or 33 of FW_2 or in position 45 of CDR_2 .